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Effect of plant materials against *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

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ABSTRACT

Laboratory experiments were conducted during 2016-17 to evaluate the efficacy of different plant materials against *Tribolium castaneum* Herbst. The treatments used in the study were neem, turmeric, ajwain, mint and untreated control. All treatments were replicated three times. All the plant materials were applied at the rate of 2% in 200 grams of wheat. Ten adult *T. castaneum* were

released in individual replication. The data was collected on adult mortality after 24 hours, 48 hours and seven days of the release. Population fluctuation data was collected on monthly basis, whereas, weight loss was recorded at the end of experiment. Results of the study indicated promising effect of all the materials to cause mortality of *T. castaneum* in wheat after one week as 100% mortality was recorded in mint, followed by turmeric (73.30%), neem and ajwain (43.30%). However, after one week, population of *T. castaneum* showed buildup in different plant extracts except mint, where 100% mortality was recorded. The highest population growth of *T. castaneum* was recorded in ajwain (186.0±24.33) at the end of experiment i.e., after three months, whereas the lowest population was observed in neem (17.33±8.45), followed by turmeric and control. The highest and lowest overall loss of wheat was observed in ajwain (18.05%) and neem (7.55%), respectively. Moreover, ajwain also suffered maximum grain losses (36.50%), followed by control (28.50%) and turmeric (17.20%). Mint treated wheat showed the minimum grain losses (2.09%) of wheat by the *T. castaneum*. It is concluded from the study that mint was the most effective botanical against *T. castaneum* and should be exploited further on large scale to safeguard the grains from losses caused by the *T. castaneum.*

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the principal food grain of Pakistan that occupies the largest area under single crop. Wheat is cultivated on a large area of land than any other cereal crop. It is considered as the most staple food for human beings. Wheat is one of the most important cash crops which is used in World trade on large scale than all other cash crops combined together (Curtis and Macpherson, 2002). It contributes ten percent to the value-added in agriculture and 2.10% to the national GDP. During 2014-15, area under wheat cultivation was 0.918 mha with production of 25.4 mt and yield of 2,775 kg ha-1 (GoP, 2015). Wheat has important ingredients that make it a nutritious food with fifty-five percent carbohydrates and twenty percent food calories (Kumar et al., 2011). An increase in human population of the world led to a number of problems, particularly with respect to food losses. Storage pests damage 10 to 40% of stored agricultural food products throughout the world (Raja et al 2001). Millions of dollars are lost every year due to stored grain insect pests (Flinn et al., 2003).

Losses due to an attack of insect pests are the most severe problem in store grain houses, particularly in the under developing countries poor sanitation and use of improper storage facilities all support the attack of pest (Talukder *et al*., 2004). Several storages of agricultural products are attacked by more than six hundred different species of beetle pests. More than seventy species of moths and approximately 355 species of mites cause quantitative and qualitative losses in wheat (Rajendran, 2002).

Tribolium castaneum Herbst (Coleoptera: Tenebrionidae) is one of the most extensive and devastating pests in storage products, feeding on different grain and cereal products (Weston and Rattlingourd, 2000; Mishra *et al*., 2012). The larvae obliterate 12.5-14.60% of the weight of single seeds and in the course of their growth; 88 grains are attacked per larva (Ali *et al.,* 2011). It primarily attacks milled grain products such as cereals and flour and is known as bran bug. It does not feed on intact grains and causes considerable losses to flour (Li and Arbogast, 1991). As being a major pest of wheat, red flour beetle has a maximum rate of population growth noted for any pest of stored products (Ahmed *et al.,* 2010).

Red flour beetle is not only deteriorating the quality of food grains but also cause significant quantitative loss of stored food products. Insect pests usually cause 5-10% losses of stored food grains worldwide. However, these losses could be as high as 50% in tropical countries during summer season when conditions of high temperature and relative humidity prevail (Raja *et al.,* 2001; Ahmad and Ahmad, 2002).

Insecticides cause serious problems such as environmental pollution, toxic residues in grain storage, increased cost of use and insect resistance (Collins *et al*., 2005; Jovanovic *et al*., 2007). Therefore, it requires an urgent need to widen safe alternatives pesticides have low cost, suitable to use and environmentally friendly. Mostly, synthetic insecticides are used for the management of *T. castaneum* in the warehouses (Gonçalves *et al*., 2007). However, injudicious and overuse of pesticides has detrimental effects on the environment and non-target organisms (Agarwal *et al.,* 2007).

Grain managers tend to use only chemical alternatives to control pests in food storage, but as consumers become less tolerant of pesticide residues in food, interest in non-chemical methods is increasing (Flinn and Hagstrum, 2001). Therefore, due to the strict requirements for the safe use of synthetic pesticides on or near food, the choice of pesticides is very limited (Mohan *et al*., 2010). In recent times, the trend has been shifted towards the use of plant materials as a very cheap and cost-effective source for the management of noxious stored grain pests (Adedire and Lajide, 2003; Oni and Aialleke, 2008; Akinkurolel *et al*., 2009). Plant material with insecticidal properties is one of the most important locally available, biodegradable and inexpensive methods for controlling stored grain pests (Mishra *et al*., 2012). The main advantages of botanicals include easy access to farmers, small-scale industries, and lower prices (Nikkon *et al*., 2009). The use of plant insecticides to protect stored products is promising, primarily because it is possible to control the environmental conditions inside the storage unit and maximize the insecticidal effects of these areas (Guzzo

et al., 2006). Many plant materials have demonstrated insecticidal activity against coleopteran pests of stored grain including *T. castaneum* (Padín *et al*., 2000; Tsao *et al*., 2002; Al-Jabr, 2006; Juan Hikawczuk *et al*., 2008; Benzi *et al*., 2009 Arora *et al*., 2011).

Compared with conventional insecticides, plant insecticides should have less damage to human and environmental health. Many of them degrade rapidly and do not accumulate in the human body and the environment, while others have very strong pest specificity and almost no damage to other organisms. Plant powders, extracts, and vegetable oils have been used to decrease the post-harvest losses of cereals and grain (Ofuya *et al*., 2007; Nwaubani and Fasoranti, 2008). Neem plants contain several thousands of chemical constituents having insecticidal properties. Enormous active ingredients are found in various parts of the neem but mostly have been found concentrated in seed kernels (Mondal and Mondal, 2012). The extracted essential oil from turmeric (*Curcuma longa* L.) leaves was found to be insecticidal with both contact and fumigant toxicity potentials (Tripathi, 2002). Mint (*Mentha piperita*) oils decreased the fecundity of female moths of *S. littoralis* (Klingauf *et al.,* 1982). Moreover, mint oils have huge effect on different growth stages of the cutworms and react as a stomach and contact poisons on the larvae of cutworms (Fallatah, 2003).

Therefore, the aim of this study was to evaluate the efficacy of different plant material in the management of *T. castaneum*. This approach was allowed discovering natural and safer agents for the development of bio-rational insecticides.

Objectives of the study

i. To determine the effect of different plant materials against *T. castaneum* under laboratory conditions
ii. To determine the weight loss of wheat due to the infestation of *T. castaneum*

2. MATERIALS AND METHODS

Experimental Area

The experiment was conducted in the Stored Grain Laboratory, Department of Entomology, Faculty of Crop Protection, Sindh Agriculture University Tando Jam.

Insect Collection

The culture of *Tribolium castaneum* was obtained from Grain Storage Research Laboratory, Karachi University.

Insect Rearing

Tribolium castaneum population was reared on the pure wheat flour under controlled temperature (28±5°C) and 75±5% R.H.

Plant Materials

Four plant species were selected as pest protectants i-e. Neem (*Azadirachta in*dica A. Juss), Turmeric (*Curcuma longa* L.*)*, Ajwain (*Carum copticum* Sprague) and Mint (*Mentha longifolia)*.

Extract Preparation

The plant materials were air-dried under shade in the laboratory. The dried plant materials were ground using electric blender (GEEPAS China GCG289) and sieve by muslin cloth to get the fine powder. The obtained powders of the plant materials were stored in clean plastic jars for their use in the study.

Experimental Outline

Powder of each treatment was applied at the rate of 2% into 200 grams of wheat grain (TD-1) in individual Petri dishes to observe the effect of different plant materials on the mortality of beetles. The powder was shacked thoroughly in seed for three minutes to create a homogenous mixture. In an individual Petri dish, ten freshly emerged *T. castaneum* were transferred.

Moreover, for the population fluctuation of *T. castaneum* in wheat treated with various plant materials, 2 percent concentration of each plant extract was thoroughly mixed in 200 grams of wheat in plastic jars. In each jar, twenty freshly emerged beetles of *T. castaneum* was transferred, and covered with muslin cloth and tighten with elastic rubber band to confirm the aeration and restrict the insects within the jars. Moreover, the moisture content of wheat was determined following the procedure of Lee (2012). Initially, weight of wheat was determined using the electric balance and then wheat grains were dried in an oven till no more loss in grain was noticed. The moisture content was determined by using the following formula:

$$\text{Moisture Content \%} = \frac{\text{Original Weight} - \text{Final weight after heating}}{\text{Original weight}} \times 100$$

The experiment was organized in a Completely Randomized Design (CRD) and all the treatments were replicated thrice.

Determination of Pest Damage Parameters

The toxicity of the plant's extracts was recorded after 24 hours, 48 hours and 7 days, the numbers of live and dead *T. castaneum* were counted. Moreover, for population fluctuation of *T. castaneum*, observations on number of live beetles were taken at monthly intervals for three consecutive months. At the end of three months, weight loss in the individual treatments was also recorded. The data recorded for various observations were then analyzed using SAS 9.3 statistical software and the means with significant differences were compared using the Least Significant Difference (LSD) at 0.05 significant level.

3. RESULTS

The aim of the present study was to examine the effect of different plant extracts on *Tribolium castaneum* in wheat under laboratory conditions. All the botanical materials used exhibited the potential to cause mortality of *T. castaneum* and their effectiveness against *T. castaneum* increased with the passage of time. The detailed results are discussed below:

Effect of botanical extracts on the mortality of *T. castaneum* at different intervals

The mortality rate of *T. castaneum* in wheat due to the application of various plant extracts i.e., neem, turmeric, ajwain and mint after 24 hours, 48 hours and 1 week of their application are given in Table 1. According to results, all the plant materials showed their effectiveness against the beetles in wheat. Therefore, a significant difference ($F = 7.79$, $P < 0.004$) was recorded among various plant materials to elicit mortality of *T. castaneum* after 24 hours of application. After 24 hours, the highest mortality was recorded in mint (4.00±0.58) and followed by turmeric (2.67±0.88), whereas, no mortality was recorded in control. Equal mortality (2.33±0.33) of *T. castaneum* was recorded in neem and ajwain treatments. A rise in mortality was recorded after 48 hours of application as the significantly higher ($F = 10.094$, $P < 0.0012$) mortality of *T. castaneum* was observed in mint (6.33±0.67), followed by neem (4.00±0.58), turmeric (3.33±0.67) and ajwain (3.33±0.88). After one week of application, mortality of *T. castaneum* increased in mint treatment that caused death of all the treated insects. The mortality of *T. castaneum* observed in neem, turmeric, and ajwain was 5.33±0.33, 4.33±0.33 and 4.00±0.58, respectively. Therefore, a significant difference ($F = 59.80$, $P < 0.001$) was recorded in the mortality of *T. castaneum* due to the application of various plant extracts.

Overall at the end of the week, the highest mortality percentage of *T. castaneum* was recorded in mint that caused 100% mortality. The mortality percentage recorded in neem, turmeric, ajwain, and control at the end of week was 53.30%, 43.30%, 40.00%, and 10.00%, respectively.

Table 1 Effect of different botanical extracts on the mortality of *T. castaneum* at different intervals under laboratory conditions.

Treatment	Pre-treatment Population	24 hours	48 hours	1 Week	Mortality %
Neem	10	2.33±0.33b	4.00±0.58b	5.33±0.33b	53.30%
Turmeric	10	2.67±0.88ab	3.33±0.67b	4.33±0.33bc	43.30%
Ajwain	10	2.33±0.33b	3.33±0.88b	4.00±0.58c	40.00%
Mint	10	4.00±0.58a	6.33±0.67a	10.00±0.00a	100.00%
Control	10	0.00±0.00c	0.33±0.33c	1.00±0.58d	10.00%

Table 2 Effect of different plant extracts on the population fluctuation of *T. castaneum* in wheat under laboratory conditions.

Treatment	Pre-treatment population	1st month	2nd month	3rd month
Neem	20	20.33±2.19b	55.67±4.33b	70.33±4.98b
Turmeric	20	16.67±1.33b	57.00±3.46b	80.67±5.36b
Ajwain	20	18.33±2.03b	52.00±5.69b	89.33±6.12b
Mint	20	0	0	0
Control	20	30.67±2.91a	94.67±5.46a	172.33±7.22a

Effect of botanical extracts on the population fluctuation of *T. castaneum*

Results for the population fluctuation of *T. castaneum* in wheat treated with various plant extracts are given in Table 2. A significant difference was recorded in the population of *T. castaneum* in different plant extracts treated wheat after first month ($F = 8.29$, $P < 0.0078$), second month ($F = 17.20$, $P < 0.0008$) and third month ($F = 61.10$, $P < 0.001$) of observations. After one month, no

population was recorded in wheat treated with mint; however, a slow rise in the population of *T. castaneum* was recorded in the remaining treatments. After one month, the highest mean population was recorded in control (30.67±2.91 beetles), followed by neem (20.33±2.19 beetles) and ajwain (18.33±2.03 beetles). In the remaining two months of observations, steady growth was observed in the neem, turmeric and ajwain treatments; however, rapid growth was recorded in the control. Accordingly, at the end of month three, the highest population of *T. castaneum* was recorded in control (172.33±7.22 beetles), whereas, the beetle population observed in ajwain, turmeric, and neem treatments were 89.33±6.12 beetles, 80.67±5.36 beetles, and 70.33±4.98 beetles, respectively.

Weight loss of wheat mixed with different plant extracts by *T. castaneum*

The weight loss in wheat due to the feeding of *T. castaneum* under the effect of plant extracts mixed with wheat is given in Table 3. Results indicated that overall weight loss in various treatments differs significantly ($F = 81.80$, $P < 0.001$) due to feeding of *T. castaneum* as significantly the highest weight loss at the end of experiment was observed in (37.67 grams or 18.83%), followed by ajwain (24.67 grams or 12.33%) and turmeric (17.00 grams 8.50%) treatments. Moreover, the lowest overall weight loss was recorded in mint (11.00 grams or 5.50%)) and neem (14.23 grams or 7.12%) treatments. In continuation of overall weight loss of weight, the highest grain weight loss of wheat due to infestation of *T. castaneum* was also recorded in control (54.33 grams or 27.17%), followed by ajwain treatment (42.00 grams or 21.00%). The lowest grains weight loss was observed in mint treatment (15.33 grams or 7.67%), followed by turmeric (22.00 grams or 11.00%) and neem (22.17 grams or 11.08%) treatments. Accordingly, the various treatments showed a significant ($F = 68.90$, $P < 0.001$) difference in grain weight loss due to the infestation of *T. castaneum* and influenced by the addition of plant materials.

Table 3 Weight loss of wheat mixed with different plant extracts by *T. castaneum* under laboratory conditions

Treatment	Initial weight (g)	Final weight (g)	Grain weight (g)	Overall weight loss (grams / %)	Grain weight loss (grams / %)
Neem	200 (g)	185.77±0.19ab	177.83±1.48b	14.23 (7.12%)	22.17 (11.08%)
Turmeric	200 (g)	183.00±58b	178.00±1.15b	17.00 (8.50%)	22.00 (11.00%)
Ajwain	200 (g)	175.33±2.03c	158.00±2.08c	24.67 (12.33%)	42.00 (21.00%)
Mint	200 (g)	189±0.58a	184.67±1.76a	11.00 (5.50%)	15.33 (7.67%)
Control	200 (g)	162±1.45d	145.67±2.91d	37.67 (18.83%)	54.33 (27.17%)

Moisture percentage of wheat before and after the experiment

Percentage moisture content in wheat was measured before and after the experiment and the results are given in Table 4. The moisture content in wheat at the start of the experiment in all treatment was 6%. However, due to the addition of plant extracts and damage caused by *T. castaneum,* at the end of study, the maximum moisture gain (2.91%) was recorded in control, followed by ajwain (2.11%) and turmeric (1.16%). Moreover, the lowest moisture content was recorded in mint (5.06%) that showed a decline in moisture of 0.96% and followed by neem (5.63%) in which moisture decline of 0.37% was recorded.

Table 4 Moisture percentage of wheat before and after the experiment under laboratory conditions

Treatment	Initial moisture %	Final moisture %	Change %
Neem	6	5.63	-0.37
Turmeric	6	7.16	1.16
Ajwain	6	8.11	2.11
Mint	6	5.04	-0.96
Control	6	8.91	2.91

4. DISCUSSION

Laboratory studies were conducted in the Department of Entomology to evaluate the effect of various botanical pesticides against *T. castaneum* in wheat regarding its damage and mortality. The botanical pesticides in the experiment were neem, turmeric, ajwain, and mint in the powder form. The data was recorded regarding the mortality, population fluctuation and weight loss of wheat caused by *T. castaneum,* after the application of the botanical mentioned above. Mortality data showed that mint caused the 100% mortality of the targeted *T. castaneum*, followed by neem and turmeric, whereas the lowest mortality was recorded in control,

followed by ajwain. Moreover, population fluctuation of *T. castaneum* in wheat mixed with plant extracts indicated that the maximum population growth of pest was recorded in control, followed by ajwain, turmeric, and neem treatments. No growth was observed in mint treatments where all the individuals of *T. castaneum* died after one week of experiment. In continuity with mortality and population data, the lowest weight loss of wheat was recorded in mint treated wheat, whereas, the highest overall and grains weight loss was recorded in control, followed by ajwain, neem, and turmeric.

Previous studies also confirmed the significant role of various plant extracts in the management of *T. castaneum*, hence, our stored wheat face minimum losses from it. Farhana *et al*. (2006) confirmed the potential of plant extracts of coriander, ajwain, and fenugreek against adults of *T. castaneum* with LD_{50} values of 316.17, 243.58 and 271.45, respectively. Therefore, the fenugreek extracts were found to be most toxic against the beetles followed by ajwain and coriander. Moreover, repellence experiments confirmed that fenugreek significantly showed more deterrence activity against *T. castaneum* followed by coriander and ajwain. Tripathi *et al*. (2009) also mentioned that cardamom, turmeric, and ginger possess the contact toxicity against the *T. castaneum*, however, a better fumigant toxicity of cardamom powder was recorded against it. Clove powder showed 100% repellency against *T. castaneum* at a dose of 1.5 g/50 g, whereas, no new progeny was observed at the dose of 5 g/100 g for all three plants i.e., cardamom, cinnamon and clove. Moreover, 1:1 mixture of clove and large cardamom exhibited both repellent and inhibited progeny development in *T. castaneum*. Many studies also showed the potential of neem to be used not only as the repellent against the stored grain pests especially *T castaneum,* but also inhibit the growth, development and in many cases toxic properties against it (Adarkwah *et al.,* 2010; Iqbal *et al.,* 2010).

Moreover, Iqbal *et al.* (2010) also confirmed the potential use of seven plant species against the *T. castaneum* and found that extract of *Acorus calamus* and *A. indica* exhibited more than 40% average repellency. Bilal *et al.* (2015) also investigated the potential of oils obtained from the seeds of citrus on various developmental stages of *T. castaneum.* They found that LC_{50} values of different oils i.e., *Citrus jambhiri*, *C. reticulate*), *C. reticulate* and *C. sinensis* against adult *T. castaneum* were 5.47%, 7.70%, 10.79%, and 11.79%, respectively. Whereas, the LC_{50s} values of the same oils against larvae were calculated as 11.27%, 17.31%, 106.85%, and 111.20%, respectively. The lowest LT_{50} against adults and larvae of beetle were obtained at 6.89 h and 4.06 h, respectively when they were treated with *C. jambhiri*. Joel (2015) studies also confirmed that extracted powders of *A. indica, Lawsonia inermis, A. senegalensis* and *Hyptis suaveolens* showed pesticidal properties against *T. castaneum.* Accordingly, the least number of damaged seeds after 28 days of applications was observed in *A. indica* treatments, followed by *L. inermis* at the concentration of 20 grams per 250 grams of seeds. Similarly, the highest percentage mortality of *T. castaneum* was recorded in 20 g concentration of *A. indica* (53.33%), followed by the same concentration of *L. inermis* (33.33%), whereas, the lowest mortality was recorded in *H. suaveolens* (16.66%) and *A. senegalensis* (20.00%). At the end of experiment (after week six), the highest weight loss was recorded in untreated control (16.36%) seeds, whereas, the lowest damaged seeds were observed in *A. indica* treatment (0.75%), followed by seed treated with *L. inermis* (2.02%), both at 20 g concentration.

Therefore, results of all the above-mentioned studies confirmed that many plant extracts have potential to be used as potential insecticides against stored grain pests and especially against *T. castaneum.* The plant extracts evaluated in the studies showed various properties ranging from repellency to the mortality of insect pests. In this study, mint, turmeric, and neem cause the promising mortality with 100% mortality recorded in mint. Accordingly, all these plant extracts also retard the growth of the *T. castaneum,* as no rapid reproduction and growth were recorded in all the treatments of the plants used in the study. In comparison, highest growth and development of the pest were recorded in the control.

5. SUMMARY, CONCLUSIONS, AND SUGGESTIONS

Summary

Among the pests, *T. castaneum* is one of the serious pests of wheat worldwide. Mostly synthetic fumigants are used to control the populations of stored grain pests, however, all such fumigants are also harmful to humans due to direct consumption of grains by humans. Therefore, in present years research has been focused to use safe control methods for the management of these pests including the use of plant materials. Therefore, this study was undertaken to evaluate the insecticidal effect of different plant materials against *T. castaneum* under laboratory conditions. The plants used in the study were neem, turmeric, ajwain, and mint. Results of the study indicated promising effect of all the materials to cause mortality of *T. castaneum* in wheat after one week with 100% mortality recorded in mint, followed by neem (53.30%), turmeric (43.30%), and ajwain (40.00%). However, after one week of the exposure, a gradual increase was recorded in the population of *T. castaneum* in all the treatments, except mint, where 100% mortality was recorded. At the end of third month of study, among the treatments, the highest population growth of *T. castaneum* was recorded in ajwain (89.33±6.12), followed by turmeric (80.67±5.36) and neem (70.33±4.98). The maximum population of *T. castaneum* at the end of experiment (three months) was recorded in control (172.33±7.22). In view of the mortality and population development among

various treatments, overall the highest and lowest weight losses of wheat were recorded in control (18.83%) and mint (5.50%) treatments, respectively. Similarly, *T. castaneum* caused the maximum weight loss of grains in control (27.17%), whereas, the minimum grain weight loss was recorded in mint treatment (7.67%).

Conclusions

The following conclusions have been drawn from the study undertaken.

1. All the plant materials showed toxic properties against the *T. castaneum.*
2. 100% mortality was recorded in the mint treatment.
3. Control exhibited the maximum population growth of *T. castaneum*, whereas, the lowest population growth was recorded in neem.
4. The maximum and minimum weight losses were recorded in control and mint treatments, respectively

Recommendations

Based on the findings of the study, following recommendation is suggested:

1. Mint and neem should be used in stored grains against *T. castaneum.*
2. Further studies should be conducted on the use of various concentrations of the tested plant materials to determine the optimum dose and time to cause maximum mortality of *T. castaneum*; thus, the lowest damage to wheat grains.

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